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13. ABSTRACT (Maximum 200 words)
We investigated the potential catalytic activity of the Zn(II) containing antibody against a variety of ester and phosphomonoester and diester substrates. They were designed on the basis of their structural resemblance to the 43C9 hapten. None of the materials proved to be substrates for the Zn(II) containing antibody and the project was terminated.

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I wish to make the attached Annual Progress Report serve as part of my **Final Report**.

Date: 4-22-97

Stephen J. Benkovic
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ANNUAL PROGRESS REPORT

GRANT#: N00014-91-J-1593

PRINCIPAL INVESTIGATOR: Stephen J. Benkovic

INSTITUTION: The Pennsylvania State University

GRANT TITLE: The Use of Combinatorial Heavy & Light Chain Libraries and Site Specific Mutagenesis to Create Antibody Biosensors for Metal Ions

REPORTING PERIOD: June 1, 1993 - March 31, 1994

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OBJECTIVE: To construct F_{ab} fragments that possess metal ion binding ligands in juxtaposition to the antigen combining site, so that the binding of both antigen and metal produces observable chemical or spectral changes in the antigen.

APPROACH: A recursive protocol will be used to create the required F_{ab} fragments. i) Antibodies with the desired binding functions will be induced with the appropriate immunogen and F_{ab}s obtained from recombinant libraries, ii) F_{ab}s will be screened for antigen binding and those with appropriate affinities isolated, sequenced and overexpressed in *E.coli*; iii) The structures of selected F_{ab}s will be deduced from modeling (in collaboration with Drs. Getzoff and Roberts, Scripps Clinic) and amino acid motifs required for metal ion binding will be introduced by site specific mutagenesis; iv) Purified F_{ab}s will be tested for their metal ion affinity and response to a transducing substrate; v) improvements in the binding and catalytic properties of the F_{ab} will be sought by additional rounds of mutagenesis and/or chain shuffling with the original light or heavy chain libraries.

ACCOMPLISHMENTS (last 12 months): We have selected the antibody, 43C9, which efficiently catalyzes the hydrolysis of aromatic ester and anilides as a monoclonal antibody, and have converted it to a single chain form, 43C9SCA. The expression vector, pJS118 (documented in our previous annual progress report) permits secretion of the SCA from *E.coli* and serves as a vehicle for site specific mutagenesis.

A Zn(II) bind site has been introduced into catalytic antibody 43C9, which accelerates the hydrolysis of an aromatic amide by a factor of 10^6 over the rate of the uncatalyzed reaction. The binding site was constructed using a computer model of the 43C9 Fv fragment and the ligand field was based on that found in carbonic anhydrase. In 43C9, the site consisted of three histidine residues: an existing histidine at position H35 as well as two site-directed mutations (N-H33-H and Y-H95-H). The mutant 43C9 antibody binds Zn(II) with a K_D value of 1.54 ± 0.09 μM and the binding of Zn(II) quenches the intrinsic antibody fluorescence by 85%, allowing this antibody to function

as a biosensor for Zn(II). The large extend of fluorescence quenching by the diamagnetic Zn(II) ion may result from changes in hydrogen bonding between one of the putative ligands (His H35) and Trp H47. This site was highly selective for Zn(II) and the binding affinities for Mg(II), Ca(II), Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Cd(II) were at least two orders of magnitude lower. By measuring the absorbance spectrum of p-nitrophenol bound to the mutant antibody in the presence of increasing concentrations of Zn(II), we demonstrated that the antibody can simultaneously accommodate both Zn(II) and *p*-nitrophenol and that they are in sufficiently close proximity to interact electronically.

SIGNIFICANCE: We have our first evidence that we have constructed a metal binding antibody with high metal ion specificity but also capable of binding an external ligand.